Exhibit O

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TRANSGENDER HEALTH

Early Hormonal Treatment Affects Body Composition and Body Shape in Young Transgender Adolescents

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ABSTRACT

Background: Transgender adolescents aspiring to have the body characteristics of the affirmed sex can receive hormonal treatment. However, it is unknown how body shape and composition develop during treatment and whether transgender persons obtain the desired body phenotype.

Aim: To examine the change in body shape and composition from the start of treatment with gonadotropin-releasing hormone agonists (GnRHa) until 22 years of age and to compare these measurements at 22 years with those of age-matched peers.

Methods: 71 transwomen (birth-assigned boys) and 121 transmen (birth-assigned girls) who started treatment from 1998 through 2014 were included in this retrospective study. GnRHa treatment was started and cross-sex hormonal treatment was added at 16 years of age. Anthropometric and whole-body dual-energy x-ray absorptiometry data were retrieved from medical records. Linear mixed model regression was performed to examine changes over time. SD scores (SDS) were calculated to compare body shape and composition with those of age-matched peers.

Outcomes: Change in waist-hip ratio (WHR), total body fat (TBF), and total lean body mass (LBM) during hormonal treatment. SDS of measures of body shape and composition compared with age-matched peers at 22 years of age.

Results: In transwomen, TBF increased (+10%, 95% CI = 7−11) while total LBM (−10%, 95% CI = −11 to −7) and WHR (−0.04, 95% CI = −0.05 to −0.02) decreased. Compared with ciswomen, SDS at 22 years of age were +0.3 (95% CI = 0.0−0.5) for WHR, and 0.0 (95% CI = −0.2 to 0.3) for TBF. Compared with cismen, SDS were −1.0 (95% CI = −1.3 to −0.7) for WHR, and +2.2 (95% CI = 2.2−2.4) for TBF. In transmen, TBF decreased (−3%, 95% CI = −4 to −1), while LBM (+3%, 95% CI = 1−4) and WHR (+0.03, 95% CI = 0.01−0.04) increased. Compared with ciswomen, SDS at 22 years of age were +0.6 (95% CI = 0.4−0.8) for WHR, and −1.1 (95% CI = −1.4 to −0.9) for TBF. Compared with cismen, SDS were −0.5 (95% CI = −0.8 to −0.3) for WHR, and +1.8 (95% CI = 1.6−1.9) for TBF.

Clinical Implications: Knowing body shape and composition outcomes at 22 years of age will help care providers in counseling transgender youth on expectations of attaining the desired body phenotype.

Strengths and Limitations: This study presents the largest group of transgender adults to date who started treatment in their teens. Despite missing data, selection bias was not found.

Conclusions: During treatment, WHR and body composition changed toward the affirmed sex. At 22 years of age, transwomen compared better to age-matched ciswomen than to cismen, whereas transmen were between reference values for ciswomen and cismen. **Klaver M, de Mutsert R, Wiepjes CM, et al. Early Hormonal Treatment Affects Body Composition and Body Shape in Young Transgender Adolescents. J Sex Med 2018;15:251−260.**

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INTRODUCTION

Adolescents with gender dysphoria aspire to have body characteristics that are similar to those of the affirmed sex. From 12 years of age, adolescents with male-to-female gender dysphoria, referred to as transwomen, and adolescents with female-to-male gender dysphoria, referred to as transmen, can be treated with gonadotropin-releasing hormone analogues (GnRHa) to suppress puberty. Subsequently, at 16 years of age and if the person still pursues gender-affirming treatment, cross-sex hormonal treatment (CHT) is added to induce the secondary sexual characteristics of the affirmed sex.[1]

During puberty, with increasing sex steroid levels, girls develop more body fat that is deposited mainly in the gluteal and femoral region (so-called gynoid region).[2,3] This leads to a female body shape with a low waist-to-hip ratio (WHR).[3,4] Pubertal boys obtain more lean body mass (LBM) and store body fat mainly in the abdominal region (also referred to as the android region),[3] resulting in a male body shape with a higher WHR than seen in girls.[3,4] It is unknown how total and regional body fat, LBM, and body shape develop in transgender adolescents treated with GnRHa and CHT, and whether this results in a similar body composition and body shape as the affirmed sex in young adulthood.

Therefore, the 1st aim of this study was to examine the effects of treatment with GnRHa and CHT on total body and regional body fat, LBM, and body shape in adolescents with gender dysphoria. A 2nd aim of this study was to compare the achieved amount of total and regional body fat, LBM, and WHR of these transwomen and transmen at 22 years of age with reference values of the affirmed sex and to examine whether they obtained the desired body composition and body shape in young adulthood. A 3rd aim was to examine the influence of pubertal stage at start of treatment on the achieved body composition and body shape at 22 years.

METHODS

Study Design and Study Population

We retrospectively reviewed the medical records of all adolescents diagnosed with gender dysphoria (*Diagnostic and Statistical Manual of Mental Disorders, 4th Edition, Text Revision*[5]) at the VU University Medical Center (Amsterdam, the Netherlands) until December 2015. All persons who started hormonal treatment before 18 years of age, started the treatment protocol as described below,[1] had undergone whole-body dual-energy x-ray absorptiometry (DXA) during treatment, and according to their age had their medical checkups in young adulthood (>20.5 years) were eligible for this study. Data obtained during routine medical checkups on anthropometry, laboratory measurements, and whole-body DXA were collected at 3 time points: start of GnRHa, addition of CHT, and result at 22 years of age (range = 20.5−23.5 years). The local ethics committee approved the study and the necessity for informed consent was waived.

Treatment Protocol

The treatment protocol, also referred to as the Dutch protocol, has been published in detail.[1] At a minimum age of 12 years and stage B2 (breast) for girls and Tanner stage G3 (genital) for boys, subcutaneous GnRHa 3.75 mg for 4 weeks was started. From 16 years of age, CHT was added with increasing doses to initiate pubertal development. Transwomen were prescribed oral 17β-estradiol starting at 5 μg per kilogram of body weight per day, which was increased by 5 μg/kg per day every 6 months until the maintenance dose of 2 mg/day was reached. Transmen used initially mixed testosterone esters (Sustanon; Organon Pharmaceuticals, Oss, The Netherlands) intramuscularly starting at 25 mg per square meter of body surface area every 2 weeks, which was increased by 25 mg/m^2 every 6 months until the maintenance dose of 250 mg every 3 to 4 weeks was achieved. When GnRHa were started after 16 years of age, CHT was added after 3 to 6 months with a start dosage of 17β-estradiol 1 mg/day or intramuscular Sustanon 75 mg/week. After 6 months, this was increased to 17β-estradiol 2 mg/day in transwomen and Sustanon 250 mg every 3 to 4 weeks in transmen. From 18 years, patients were eligible for gonadectomy, after which treatment with GnRHa ceased. From the start of treatment, patients were advised to maintain a healthy lifestyle with sportive activities and an adequate calcium intake to prevent bone loss.

Anthropometry and Whole-Body DXA

At each visit, body height, body weight, waist circumference, and hip circumference were measured. Body height was measured to the nearest 0.1 cm using a Harpenden stadiometer. Body weight was measured while the subject wore only underwear without shoes to the nearest 0.1 kg. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. Waist circumference, defined as the smallest abdominal circumference, and hip circumference, measured at the level of the trochanter major, were determined with a tape measure to the nearest 0.1 cm. From these 2 measurements, the

WHR was calculated, which was used as a measure for body shape.

Whole-body and regional body fat, LBM, and total mass were measured using DXA. Until 2002, the Hologic QDR 2000 (Hologic Inc, Bedford, MA, USA) was used. From 2002, a Hologic Delphi apparatus (Hologic Inc) was used with software version 8.26, which was updated in 2005 to version 12.3.3. In 2011, the Hologic Delphi was replaced by a Hologic Discovery 13.1 (Hologic Inc), which was updated to version 3.3 in 2012 and to version 4.5.3 in 2015. During the review of medical records, DXA scans were included when they were obtained within 4 months before or after the start of GnRHa or CHT or within 1.5 years before or after the 22nd birthday. All available DXA scans from participants were obtained and reanalyzed with the most recent software version (version 13.5.3). The android region and gynoid region were defined using the software provided by Hologic. The lower boundary of the android region coincides with the upper edge of the pelvis and the height equals 20% of the distance from the upper edge of the pelvis to the bottom of the chin. The upper boundary of the gynoid region is below the upper edge of the pelvis by 1.5 times the height of the android region. The gynoid region equals twice the height of the android region.

Statistical Analyses

STATA 13.1 (StataCorp, College Station, TX, USA) was used for statistical analyses. Linear mixed model regression analyses with observations clustered within participants were performed to examine mean changes in measurements of body composition and body shape from the start of GnRHa to 22 years of age. Linear mixed models also properly deal with missing data.[6] When outcome variables were not normally distributed, the natural logarithm was obtained for analyses. Analyses were adjusted for Tanner stage at start of treatment, and BMI at start of treatment using centered variables. Student t-tests were used to examine whether changes in body shape and body composition during GnRHa monotherapy differed from changes after the addition of CHT.

Tanner stage at start of treatment, time, and the interaction between Tanner stage and time were added to the linear mixed model regression analysis to examine the influence on the achieved body composition and body shape at 22 years of age. In transwomen, Tanner stage at start was defined by testes volume and this resulted in the following categories: less than or equal to 8 mL (early puberty), 10 to 15 mL (mid-puberty), and at least 20 mL (late puberty).[7,8] In transmen, Tanner stage at start of treatment was defined by breast development.[7] Because a small number of transmen had Tanner stage II (n = 3) or III (n = 8) at the start of CHT, the 2 groups were classified as starters in early and mid-puberty. Transmen with Tanner stage IV or V were classified as starters in late puberty. Analyses were adjusted for BMI at 22 years of age.

We calculated standard deviation scores (SDS) to compare measures of body composition and body shape in participants at 22 years of age with reference values from age-matched peers with no (treatment for) gender dysphoria, also referred to as ciswomen and cismen. In transwomen and transmen, SDS were calculated for both ciswomen and cismen in order to compare measures with the at birth assigned sex and the affirmed sex. For the mean age of start of GnRHa (15 years in both transwomen and transmen) and for the age of 22 years, age-specific reference values [4,9-12] were retrieved from literature.

RESULTS

71 transwomen and 121 transmen who started with GnRHa and CHT from 1998 through 2014 were included in the present analyses (Figure 1). The general characteristics of the participants are presented in Table 1.

Change in Body Shape and Body Composition During Treatment in Transwomen

The results of the mixed model analyses showed that in transwomen waist circumference (+8 cm, 95% CI = 5−10, $P < .001$) and hip circumference (+17 cm, 95% CI = 13−21, $P < .001$) increased and WHR decreased (−0.04, 95% CI = −0.05 to −0.02, $P < .001$). The percentage of total body fat increased (+9%, 95% CI = 8−11, $P < .001$) and thus the percentage of LBM decreased (−9%, 95% CI = −11 to −8, $P < .001$; Table 2, Figure 2). Percentage of body fat increased in the android region (+9%, 95% CI = 6−12, $P < .001$) and the gynoid region (+11%, 95% CI = 9−12, $P < .001$; Table 2). Changes in body composition and body shape measurements were not different after adjustment for Tanner stage at start of treatment, or BMI at start of treatment.

Change in Body Shape and Body Composition During Treatment in Transmen

Transmen showed increases in waist circumference (+6 cm, 95% CI = 4−8, $P < .001$), hip circumference (+5 cm, 95% CI = 2−7, $P < .001$), and WHR (+0.03, 95% CI =

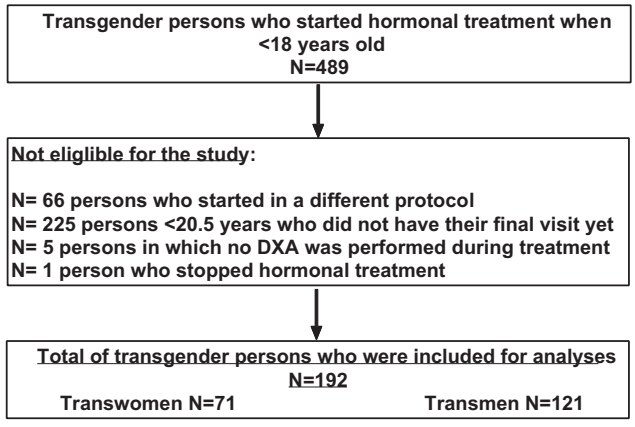

Figure 1. Flowchart of participant inclusion process. DXA = whole-body dual-energy x-ray absorptiometry.

Table 1. Characteristics of adolescents who started treatment with GnRHa and CHT at the VU University Medical Center from 1998 through 2014*

	Transwomen	Transmen
Adolescents	71	121
Age at start of GnRHa (y)	14.5 ± 1.8	15.3 ± 2.0
Age at start of CHT (y)	16.4 ± 1.1	16.9 ± 0.9
Ethnicity, %		
Caucasian	98	94
Asian	1	2
Black American	1	2
Occurrence of menarche, %†	–	84
BMI at start (kg/m^2)	19.8 (18.0–22.0)	20.6 (19.1–23.1)
Duration of GnRHa monotherapy (y)	2.1 (1.0–2.8)	1.0 (0.5–2.9)
Duration of GnRHa + CHT (y)	3.1 (2.5–3.6)	2.4 (2.0–3.1)
Duration of CHT monotherapy (y)	2.8 (1.6–3.4)	3.0 (1.9–3.4)
E2 level at start of GnRHa (pmol/L)	50 (20–79)	112 (70–202)
E2 level at start of CHT (pmol/L)	25 (20–31)	28 (23–36)
E2 level at 22 y of age (pmol/L)	121 (81–154)	70 (43–135)
T level at start of GnRHa (nmol/L)	10.0 (4.3–14.0)	1.0 (1.0–1.3)
T level at start of CHT (nmol/L)	1.0 (1.0–1.0)	1.0 (1.0–1.0)
T level at 22 y of age (nmol/L)	1.0 (0.8–1.0)	16.0 (8.8–37.0)
SDS at start vs ciswomen		
BMI	0.1 (−0.1 to 0.4)	0.4 (0.4–0.8)
Waist	0.6 (0.3–0.9)	0.6 (0.3–0.8)
Hip	0.0 (−0.2 to 0.2)	1.1 (0.1–0.5)
WHR	0.9 (0.6–0.9)	0.2 (0.0–0.3)
Total body fat	−0.9 (−1.0 to −0.7)	−0.1 (−0.2 to 0.1)
Lean body mass	2.5 (2.0–2.8)	1.3 (1.0–1.5)
SDS at start vs cismen		
BMI	0.4 (0.1–0.7)	0.7 (0.7–1.1)
Waist	0.1 (−0.2 to 0.4)	0.1 (−0.2 to 0.3)
Hip	0.3 (0.0–0.6)	0.7 (0.4–0.8)
WHR	−0.2 (−0.3 to 0.0)	−1.0 (−1.0 to −0.7)
Total body fat	1.6 (1.5–1.8)	2.0 (1.9–2.0)
Lean body mass	−0.7 (−0.9 to −0.6)	−1.1 (−1.1 to −1.0)

BMI = body mass index; CHT = cross-sex hormonal treatment; E2 = estradiol; GnRHa = gonadotropin-releasing hormone analogues; SDS = SD score; T = testosterone; WHR = waist-to-hip ratio.
*Data are presented as number, mean ± SD, median (interquartile range), or SDS (95% CI).
†Data were missing for 8% of transmen.

0.01−0.04, $P < .002$). Percentage of total body fat decreased (−3%, 95% CI = −4 to −2, $P < .001$), so percentage of LBM increased (+3%, 95% CI = 2−4, $P < .001$; Table 2, Figure 2). Percentage of body fat decreased in the gynoid region (−5%, 95% CI = −6 to −3, $P < .001$) with no change in the android region (+1%, 95% CI = 0−3, $P = .18$; Table 2). Changes in body composition and body shape measurements were not different after adjustment for Tanner stage at start of treatment, or BMI at start of treatment.

Body Shape and Body Composition at 22 Years Compared With Peers

SDS of body shape and body composition in transgender persons for both ciswomen and cismen are presented in Table 3.

Influence of Tanner Stage at Start on Body Shape and Body Composition at 22 Years

Transmen who started CHT in early or mid-puberty had a higher WHR than transmen who started treatment in late puberty. Transwomen tended to have a lower WHR starting in early or mid-puberty than those who started in late puberty. No differences in percentage of total body fat or percentage of total LBM were found across Tanner stages at start of treatment in transwomen and transmen (Table 4).

DISCUSSION

This study of 71 transwomen and 121 transmen shows that measurements of body shape and body composition change

Table 2. Measurements of body shape and body composition at the start of GnRHa, the start of CHT, and at 22 years of age in transwomen (n = 71) and transmen (n = 121)

	Start of GnRH	Start of CHT	22 y of age
Transwomen			
Body weight (kg)	58 (56–61)	66 (63–69)	76 (71–82)
BMI (kg/m^2)	20.2 (19.4–20.9)	21.3 (20.5–22.0)	23.2 (21.6–24.8)
Waist circumference (cm)*	71 (69–73)	75 (73–77)	79 (77–82)
Hip circumference (cm)*	89 (87–91)	95 (93–97)	106 (102–110)
WHR*	0.81 (0.79–0.82)	0.79 (0.78–0.80)	0.77 (0.75–0.79)
Body fat			
Total body (%)*	25 (23–26)	31 (29–32)	34 (32–36)
Android (%)*	23 (21–25)	28 (26–31)	32 (28–36)
Gynoid (%)*	29 (27–30)	36 (34–38)	40 (38–42)
Lean body mass			
Total body (%)*	75 (74–77)	69 (68–71)	66 (64–68)
Transmen			
Body weight (kg)	58 (56–61)	63 (60–65)	69 (66–71)
BMI (kg/m^2)	21.6 (20.9–22.3)	22.5 (21.7–23.2)	23.9 (23.0–24.7)
Waist circumference (cm)*	71 (69–72)	73 (71–74)	77 (75–79)
Hip circumference (cm)*	92 (90–93)	95 (93–97)	96 (94–99)
WHR*	0.77 (0.76–0.78)	0.76 (0.75–0.77)	0.80 (0.78–0.82)
Body fat			
Total body (%)*	30 (29–31)	33 (32–35)	27 (26–28)
Android (%)*	29 (27–30)	33 (32–35)	30 (28–32)
Gynoid (%)*	36 (35–37)	39 (38–40)	31 (30–33)
Lean body mass			
Total body (%)*	70 (69–71)	67 (66–68)	73 (72–74)

BMI = body mass index; CHT = cross-sex hormonal treatment; GnRHa = gonadotropin-releasing hormone analogues; WHR = waist-to-hip ratio.
*Percentages of missing data for anthropometrics were 11% in transwomen and 18% in transmen for start of GnRHa, 10% in transwomen and 13% in transmen for start of CHT, and 71% in transwomen and 76% for visit at 22 years of age. For measurements of body composition examined by whole-body dual-energy x-ray absorptiometry, percentages of missing data were 12% in transwomen and 11% in transmen for start of GnRHa, 36% in transwomen and 45% in transmen for start of CHT, and 64% in transwomen and 65% in transmen at 22 years of age.

toward the values of the affirmed sex during treatment with GnRHa and CHT. As a result of these changes, in young adult transwomen at 22 years of age, SDS for WHR, body fat, and LBM showed greater similarity to ciswomen than to cismen. In transmen at the same age, SDS for WHR, body fat, and LBM were between reference values for ciswomen and cismen. The achieved body fat and LBM at 22 years were similar across different Tanner stages at start of treatment in transwomen and transmen. However, in transmen, an earlier Tanner stage at start of treatment appeared to be associated with a closer resemblance of body shape to their affirmed sex at 22 years, and this tended to be the same in transwomen.

Compared with ciswomen and cismen in adolescence, transgender adolescents who are treated with GnRHa and CHT exhibit greater changes in body composition. A larger increase in percentage of body fat has been seen in transwomen compared with ciswomen within the same lifespan.[10] Transmen exhibit decreased percentage of body fat, whereas percentage of body fat in cismen remains stable during puberty.[10] This observation is likely explained by the fact that prepubertal girls already have more body fat than prepubertal boys, and therefore transgender persons have a different body composition at the start of hormonal treatment than age-matched peers of the affirmed sex.[13] Also, compared with adult transgender persons treated with CHT,[14,15] larger changes in body shape and body composition are seen in transgender persons who start in adolescence. Moreover, transgender persons who start treatment in adolescence have at 22 years of age a body composition that approaches the affirmed sex more closely than transgender persons who start CHT in adulthood.[15]

The appropriate moment of starting gender reassignment continues to be a topic of debate.[16] The findings of this study favor starting treatment in an early stage of puberty, because this appears to be associated with a closer resemblance of body shape to the affirmed sex at 22 years. This observation can be explained in part by the fact that in early puberty there is no distinct sex-specific body fat distribution, but also other factors can contribute to this difference. For instance, the period of gonadal suppression is generally considered a period of status quo. However, it can be postulated that a body of a transwomen

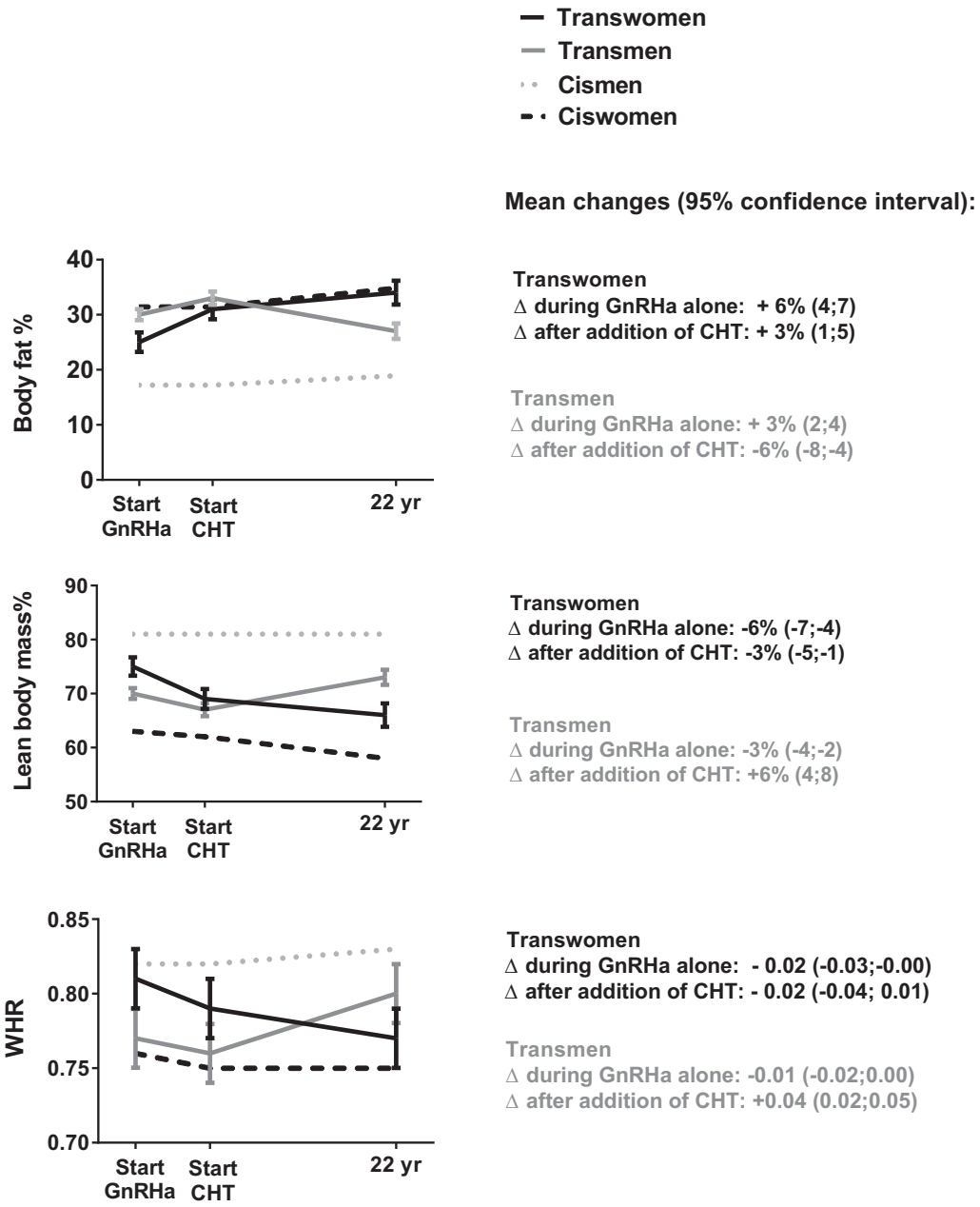

Figure 2. Changes (+95% CI) in percentage of total body fat, percentage of total body lean mass, and WHR in transwomen and transmen from the start of GnRHa to 22 years of age examined with mixed-model analyses using reference values for ciswomen and cismen. Reference data for ciswomen and cismen are shown for percentage of body fat,[10] percentage of lean body mass,[10] and WHR.[4] Mean differences between changes during GnRHa alone and changes after the addition of CHT in transwomen were +3 (95% CI −0 to 5) for percentage of total body fat, −3 (95% CI −5 to 0) for percentage of lean body mass, and 0.00 (95% CI −0.02 to 0.03) for WHR and in transmen were +9 (95% CI 7–11) for percentage of total body fat, −9 (95% CI −11 to −7) for percentage of lean body mass, and −0.04 (95% CI −0.06 to −0.02) for WHR. CHT = cross-sex hormonal treatment; GnRHa = gonadotrophin-releasing hormone analogues; WHR = waist-to-hip ratio.

in a prolonged androgen-deprived state might respond differently to estrogens than a body that has had greater androgen exposure. Although programming properties of androgens are most pronounced prenatally, during puberty programming effects have been described in the brain.[17] Therefore, other sex dimorphic organs such as body fat might also undergo androgen-induced programming that results in a lesser response to estrogens. To achieve a better outcome (ie, greater resemblance to the affirmed sex), both this phase of gonadal suppression can be modulated as the phase of gonadal suppression combined with CHT. Although in this study the period of CHT did not differ much between pubertal groups, previous

Table 3. SDS of body shape and body composition in trans persons at 22 years of age for ciswomen and cismen

	Transwomen (n = 71)		Transmen (n = 121)	
	SDS for ciswomen	SDS for cismen	SDS for ciswomen	SDS for cismen
BMI	+0.4 (−0.2 to 1.0)	+0.4 (−0.2 to 1.0)	+0.5 (0.3–0.8)	+0.6 (0.3–0.8)
Waist circumference	+1.1 (0.8–1.6)	−0.1 (−0.4 to 0.3)	+0.8 (0.5–1.1)	−0.1 (−0.4 to 0.3)
Hip circumference	+1.4 (1.0–1.9)	+1.8 (1.3–2.4)	+0.2 (−0.2 to 0.5)	+0.3 (−0.2 to 0.7)
WHR	+0.3 (0.0–0.5)	−1.0 (−1.3 to −0.7)	+0.6 (0.4–0.8)	−0.5 (−0.8 to −0.3)
Total body fat	−0.4 (−0.8 to 0.0)	+2.1 (2.1–2.3)	−1.1 (−1.4 to −0.9)	+1.8 (1.6–1.9)
Android fat	−0.1 (−0.3 to 0.3)	+0.8 (0.6–1.1)	−0.3 (−0.4 to 0.0)	+0.7 (0.5–0.9)
Gynoid fat	−0.1 (−0.4 to 0.3)	+2.1 (1.8–2.3)	−1.7 (−1.8 to −1.5)	+0.9 (0.8–1.1)
Lean body mass	+1.3 (0.8–1.8)	−1.4 (−1.5 to −1.2)	+3.0 (2.8–3.3)	−0.9 (−0.9 to −0.8)

BMI = body mass index; SDSs = SD scores; WHR = waist-to-hip ratio.

Table 4. Measurements of body shape and body composition at 22 years of age shown per category of Tanner stage at start of treatment and differences between those categories

	Tanner stages at start of treatment			Difference between Tanner stages	
Transwomen	Early puberty (n = 16)	Mid-puberty (n = 21)	Late puberty (n = 34)	ΔEarly vs mid-puberty	ΔEarly vs late puberty
Age at start (y)	13.2 (12.8–13.5)	13.3 (13.1–14.0)	15.6 (14.3–17.2)		
Duration of GnRHa monotherapy (y)	2.7 (2.4–3.1)	2.7 (2.1–3.1)	1.0 (0.6–1.6)		
Duration of GnRHa + CHT (y)	3.0 (2.6–3.6)	3.1 (2.6–3.7)	3.1 (2.3–3.5)		
BMI (kg/m^2)	23.8 (21.5–26.2)	20.9 (18.7–23.1)	24.2 (22.8–25.6)	2.9 (−0.3 to 6)	−0.4 (−3.1 to 2.3)
SDS for ciswomen	0.6 (−0.2 to 1.5)	−0.4 (−1.2 to 0.4)	2.1 (0.3–1.3)		
WHR	0.75 (0.71–0.79)	0.75 (0.69–0.80)	0.78 (0.76–0.80)	0.00 (−0.07 to 0.07)	−0.03 (−0.08 to 0.02)
SDS for ciswomen	0.0 (−0.5 to 0.5)	0.0 (−0.8 to 0.6)	0.4 (0.1–0.6)		
Total body fat (%)	33 (29–38)	30 (25–35)	34 (32–36)	3 (−4 to 10)	−1 (−6 to 4)
SDS for ciswomen	−0.2 (−0.8 to 0.4)	−0.6 (−1.4 to 0.0)	−0.1 (−0.4 to 0.1)		
Lean body mass (%)	67 (62–72)	70 (65–75)	66 (64–68)	−3 (−10 to 4)	+1 (−4 to 6)
SDS for ciswomen	1.5 (0.3–2.8)	2.3 (1.0–3.5)	1.3 (0.8–1.8)		

Transmen	Early to mid-puberty (n = 11)	Late puberty (n = 110)	ΔEarly to mid vs late puberty
Age at start (y)	12.3 ± 0.5	15.6 ± 1.7	
Duration of GnRHa monotherapy (y)	3.5 (3.4–3.9)	0.9 (0.5–2.0)	
Duration of GnRHa + CHT (y)	2.2 (2.0–2.5)	2.5 (1.9–3.2)	
BMI (kg/m^2)	22.5 (19.8–25.1)	23.9 (23.0–24.7)	−1.4 (−4.2 to 1.4)
SDS for cismen	0.1 (−0.9 to 1.1)	0.7 (0.3–1.0)	
WHR	0.85 (0.80–0.90)	0.79 (0.77–0.80)	0.06 (0.02 to 0.12)
SDS for ciswomen	0.3 (−0.5 to 1.2)	−0.7 (−1.0 to −0.5)	
Total body fat (%)	26 (21–31)	27 (26–29)	−1 (−6 to 4)
SDS for cismen	1.7 (1.3–2.0)	1.8 (1.7–1.9)	
Lean body mass (%)	74 (69–79)	73 (72–74)	1 (−4 to 6)
SDS for cismen	−0.8 (−1.1 to −0.4)	−0.9 (−0.9 to −0.8)	

BMI = body mass index; CHT = cross-sex hormonal treatment; GnRHa = gonadotropin-releasing hormone analogues; SDS = SD score; WHR = waist-to-hip ratio.

studies have shown that in adult transwomen[18]—but not in transmen[19]—a longer period of CHT establishes ongoing changes in body composition after the 1st year of treatment, which also could be applicable to body fat distribution and body shape. Therefore, one can postulate that starting CHT earlier than the current recommended age of 16[20] would improve outcomes in young adulthood.

We observed that transwomen at 22 years more closely resembled the affirmed sex regarding total body fat than transmen did. Body composition is the result of an interplay among multiple factors, including hormonal status, genetic predisposition, and lifestyle features such as diet and physical activity levels. A variance in these factors could explain this difference in treatment results between transwomen and transmen. Before the start of GnRHa, this difference was already present. Transmen had a percentage of total body fat very similar to that of ciswomen (SDS = −0.1), but transwomen had a percentage of body fat closer to that of ciswomen (SDS = −0.9) than to that of cismen (SDS = 1.6). The cause of the increased percentage of body fat and BMI in transwomen is unknown, but it can be postulated that psychological stress from gender dysphoria and an inactive lifestyle[21] could have contributed. Alternatively, the genetic predisposition, independent of hormonal treatment, of transmen could have resulted in less comparable values to their affirmed sex at 22 years than that of transwomen.[22] It can be debated whether the 46,XX karyotype[23] or other autosomal genes[24] are responsible.

As shown in Figure 2, the largest changes in transwomen were seen during GnRHa monotherapy (ie, during the suppression of testosterone). In transmen, the largest change was observed after the start of testosterone treatment. Therefore, it can be postulated that the suppression or addition of testosterone has a greater impact than the suppression or addition of estradiol. However, it is unclear whether the larger increase in body fat during GnRHa monotherapy in transwomen is due solely to the direct absence of testosterone action or the hypogonadal state itself. Indeed, also in transmen an increase in body fat was seen during GnRHa monotherapy. The larger change in body fat in transmen after the addition of testosterone could be due to the known lipolytic and anabolic effects of testosterone,[25] which in turn could be enhanced by practicing more sports or strength training in later adolescence.

This study is the 1st examining the effects of GnRHa and CHT on body shape and body composition in a large cohort of transgender adults who started treatment in their teens. A limitation of our study is the presence of missing data, which is seen more often in studies with a retrospective design when data are collected during regular patient care. However, our missing data were missing ad random and analyses between persons with missing data and persons without missing data did not indicate selection bias. Another limitation of our study is that (change in) body fat and LBM are dependent on other factors such as diet and physical activity and those were not systematically recorded.

This study provides insight into the effects of GnRHa and CHT on body shape and body composition during treatment in adolescence. Transgender adolescents starting treatment can be better informed on the extent to which their bodies will change and on what to expect of the results of such changes in young adulthood. Also, the finding that an early start of treatment in transmen results in a body shape that more closely resemble those of the affirmed sex than a late start supports early medical intervention.

The transgender persons included in this study were the first adolescents to be treated with the Dutch protocol.[1] Despite the favorable results on body shape and body composition, future research is warranted to improve current treatment protocols or devise alternative treatment protocols when GnRHa are not available.[26] Further, this study focused on body shape and body composition as important features of physical appearance, but it would also be of interest to determine the effects on body composition in the context of cardiovascular risk. For example, visceral fat seems to play an important role in the onset of insulin resistance and the metabolic syndrome,[27,28] and thus the study of changes in subcutaneous and visceral fat depots and their relation to cardiometabolic outcome could be relevant in the future.

CONCLUSION

Transwomen who started treatment with GnRHa and CHT in adolescence showed an increase in body fat with a decrease in LBM and WHR. At 22 years of age, their body shape and body composition were more similar to those of ciswomen than to those of cismen. In transmen, an increase in LBM and WHR was seen with a decrease in body fat during therapy, and at 22 years they had values between reference values for ciswomen and cismen. The achieved body fat and LBM at 22 were similar across different Tanner stages at start of treatment in transwomen and transmen. However, in transmen, start of treatment in early or mid-puberty resulted in a body shape more similar to that of the affirmed sex, and this tended to be the same in transwomen.

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